

# Supplementary

## Application of Piezo-Based Measuring System for Evaluation of Nucleic Acid-Based Drugs Influencing the Coagulation

Silju-John Kunnakattu <sup>1,2</sup>, Ludmilla Hann <sup>1</sup>, Julia Kurz <sup>1</sup>, Hanna Haag <sup>1</sup>, Stefan Fennrich <sup>1</sup>, Nicole Rauch <sup>2</sup>, Christian Schlensak <sup>1</sup>, Hans Peter Wendel <sup>1</sup>, Sandra Stoppelkamp <sup>1,†</sup>, and Meltem Avci-Adali <sup>1,\*,†</sup>

<sup>1</sup> Department of Thoracic and Cardiovascular Surgery, University Hospital Tuebingen, 72076 Tuebingen, Germany; Silju.kunnakattu@klinikum.uni-tuebingen.de (S.-J.K.); Ludmilla.hann@klinikum.uni-tuebingen.de (L.H.); Julia.kurz@klinikum.uni-tuebingen.de (J.K.); Hanna.haag@klinikum.uni-tuebingen.de (H.H.); stefan.fennrich@gmail.com (S.F.); Christian.schlensak@med.uni-tuebingen.de (C.S.); Hans-peter.wendel@med.uni-tuebingen.de (H.P.W.); Sandra.stoppelkamp@klinikum.uni-tuebingen.de (S.S); meltem.avci-adali@uni-tuebingen.de (M.A.-A.)

<sup>2</sup> Department of Micro- and Nanoanalytics, University of Applied Sciences Iserlohn, 58644 Iserlohn, Germany; Rauch.nicole@fh-swf.de (N.R.)

\* Correspondence: meltem.avci-adali@uni-tuebingen.de ; Tel.: +49-7071-29-83334; Fax: + 49-7071-29-5369

† These authors contributed equally to this work.

### Supplementary Tables

**Table S1.** Citrated blood was incubated with NaCl or various concentrations of NU172 aptamer, AD, or NS\_AD for 2 min. Afterwards, the coagulation was activated by pathromtin and CaCl<sub>2</sub> (n = 10) and measured with PIEZ and KC 1A. Using PIEZ, the clotting time (CT), viscous ( $\eta'$ ) and elastic ( $\eta''$ ) components at 300 s, and linear slopes ( $m(\eta')$  and  $m(\eta'')$ ) were measured <sup>1</sup>.

Sample	PIEZ	KC 1A	PIEZ			
	CT/s	CT/s	$\eta'$ (Pas) at 300 s	$\eta''$ (Pas) at 300 s	$m(\eta'/\text{Pa})$	$m(\eta''/\text{Pa})$
NaCl	51.8 ± 1.3	52.9 ± 2.3	$3.1 \times 10^{-3} \pm 3.8 \times 10^{-4}$	$3.0 \times 10^{-3} \pm 3.9 \times 10^{-4}$	$6.8 \times 10^{-5} \pm 1.2 \times 10^{-5}$	$8.4 \times 10^{-5} \pm 1.2 \times 10^{-5}$
0.5 $\mu\text{M}$ NU172	62.2 ± 1.9	63.3 ± 4.0	$3.7 \times 10^{-3} \pm 3.7 \times 10^{-4}$	$3.4 \times 10^{-3} \pm 6.1 \times 10^{-4}$	$7.8 \times 10^{-5} \pm 1.6 \times 10^{-5}$	$6.8 \times 10^{-5} \pm 2.2 \times 10^{-5}$
1.0 $\mu\text{M}$ NU172	78.7 ± 3.1	79.4 ± 8.0	$3.5 \times 10^{-3} \pm 2.6 \times 10^{-4}$	$3.2 \times 10^{-3} \pm 3.8 \times 10^{-4}$	$4.9 \times 10^{-5} \pm 1.3 \times 10^{-5}$	$4.2 \times 10^{-5} \pm 2.2 \times 10^{-5}$
1.5 $\mu\text{M}$ NU172	215.0 ± 10.7	211.6 ± 10.4	$2.5 \times 10^{-3} \pm 3.6 \times 10^{-4}$	$1.6 \times 10^{-3} \pm 2.6 \times 10^{-4}$	$1.1 \times 10^{-5} \pm 3.5 \times 10^{-6}$	$1.4 \times 10^{-5} \pm 2.5 \times 10^{-6}$
2.0 $\mu\text{M}$ NU172	249.1 ± 10.5	248.9 ± 14.6	$2.1 \times 10^{-3} \pm 2.8 \times 10^{-4}$	$1.4 \times 10^{-3} \pm 2.4 \times 10^{-4}$	$8.3 \times 10^{-6} \pm 3.1 \times 10^{-6}$	$1.2 \times 10^{-5} \pm 2.5 \times 10^{-6}$
1.0 $\mu\text{M}$ AD	52.7 ± 1.1	52.4 ± 1.9	$2.9 \times 10^{-3} \pm 2.0 \times 10^{-4}$	$3.5 \times 10^{-3} \pm 4.6 \times 10^{-4}$	$5.9 \times 10^{-5} \pm 6.7 \times 10^{-6}$	$9.1 \times 10^{-5} \pm 1.2 \times 10^{-5}$
1.0 $\mu\text{M}$ NS_AD	52.2 ± 1.6	51.8 ± 1.9	$3.3 \times 10^{-3} \pm 2.8 \times 10^{-4}$	$3.5 \times 10^{-3} \pm 4.1 \times 10^{-4}$	$8.0 \times 10^{-5} \pm 1.0 \times 10^{-5}$	$9.9 \times 10^{-5} \pm 2.7 \times 10^{-5}$
1.0 $\mu\text{M}$ NS	52.1 ± 1.6	51.8 ± 1.5	$3.3 \times 10^{-3} \pm 2.9 \times 10^{-4}$	$3.4 \times 10^{-3} \pm 4.0 \times 10^{-4}$	$8.2 \times 10^{-5} \pm 1.2 \times 10^{-5}$	$1.1 \times 10^{-4} \pm 2.4 \times 10^{-5}$

<sup>1</sup> clotting time (CT), viscous component ( $\eta'$ ), elastic component ( $\eta''$ ), the linear slope of the viscous or elastic component (m), thrombin aptamer (NU172), antidote (AD), nonsense antidote (NS\_AD), nonsense (NS).

**Table S2.** Citrated blood was incubated with NaCl or various concentrations of NU172 aptamer for 2 min. Then, 1.0  $\mu\text{M}$  AD was added and after 5 min the blood samples were activated with pathromtin and  $\text{CaCl}_2$ . (n = 5). The measurement was performed with PIEZ and KC 1A. Using PIEZ, the clotting time (CT), viscous ( $\eta'$ ) and elastic ( $\eta''$ ) components at 300 s, and linear slopes ( $m(\eta')$  and  $m(\eta'')$ ) were measured <sup>1</sup>.

Sample	PIEZ	KC 1A	PIEZ			
	CT/s	CT/s	$\eta'$ (Pa s) at 300 s	$\eta''$ (Pa s) at 300 s	$m(\eta'/\text{Pa})$	$m(\eta''/\text{Pa})$
NaCl	58.6 $\pm$ 2.4	59.4 $\pm$ 2.5	$3.3 \times 10^{-3} \pm 3.0 \times 10^{-4}$	$3.6 \times 10^{-3} \pm 1.4 \times 10^{-4}$	$7.2 \times 10^{-5} \pm 1.1 \times 10^{-5}$	$8.6 \times 10^{-5} \pm 3.6 \times 10^{-5}$
NU172						
1.0 $\mu\text{M}$	162.6 $\pm$ 3.6	162.0 $\pm$ 3.3	$3.3 \times 10^{-3} \pm 3.1 \times 10^{-4}$	$2.0 \times 10^{-3} \pm 3.5 \times 10^{-4}$	$2.1 \times 10^{-5} \pm 3.5 \times 10^{-6}$	$1.9 \times 10^{-5} \pm 2.1 \times 10^{-6}$
2.0 $\mu\text{M}$	268.1 $\pm$ 6.6	263.0 $\pm$ 11.5	$2.4 \times 10^{-3} \pm 1.9 \times 10^{-4}$	$1.3 \times 10^{-3} \pm 2.2 \times 10^{-4}$	$1.2 \times 10^{-5} \pm 8.5 \times 10^{-7}$	$1.3 \times 10^{-5} \pm 2.6 \times 10^{-6}$
NU172+AD						
1.0 $\mu\text{M}$ +1.0 $\mu\text{M}$	63.8 $\pm$ 1.4	66.1 $\pm$ 3.4	$3.0 \times 10^{-3} \pm 1.7 \times 10^{-4}$	$3.3 \times 10^{-3} \pm 7.5 \times 10^{-4}$	$5.6 \times 10^{-5} \pm 5.7 \times 10^{-6}$	$7.1 \times 10^{-5} \pm 1.1 \times 10^{-5}$
2.0 $\mu\text{M}$ +1.0 $\mu\text{M}$	218.6 $\pm$ 7.3	215.7 $\pm$ 34.7	$2.7 \times 10^{-3} \pm 2.4 \times 10^{-4}$	$2.2 \times 10^{-3} \pm 4.6 \times 10^{-4}$	$9.3 \times 10^{-6} \pm 3.4 \times 10^{-6}$	$1.8 \times 10^{-5} \pm 2.0 \times 10^{-5}$
NU172+NS_AD						
1.0 $\mu\text{M}$ +1.0 $\mu\text{M}$	187.3 $\pm$ 5.8	203.7 $\pm$ 15.8	$2.6 \times 10^{-3} \pm 1.9 \times 10^{-4}$	$2.7 \times 10^{-3} \pm 4.9 \times 10^{-4}$	$8.9 \times 10^{-6} \pm 6.2 \times 10^{-6}$	$1.9 \times 10^{-5} \pm 7.8 \times 10^{-6}$

<sup>1</sup>. clotting time (CT), viscous component ( $\eta'$ ), elastic component ( $\eta''$ ), the linear slope of the viscous or elastic component (m), thrombin aptamer (NU172), antidote (AD), nonsense antidote (NS\_AD).

**Table S3.** Heparinized blood was incubated with NaCl, NU172 aptamer, NS\_AD, or AD. After 2 min of incubation, the coagulation was activated with plasma cephalin and factor  $\text{X}_a$ . (n = 10). The measurement was performed with PIEZ and KC 1A. Using PIEZ, the clotting time (CT), viscous ( $\eta'$ ) and elastic ( $\eta''$ ) components at 300 s, and linear slopes ( $m(\eta')$  and  $m(\eta'')$ ) were measured <sup>1</sup>.

Sample	PIEZ	KC 1A	PIEZ			
	CT/s	CT/s	$\eta'$ (Pa s) at 300 s	$\eta''$ (Pa s) at 300 s	$m(\eta'/\text{Pa})$	$m(\eta''/\text{Pa})$
NaCl	45.1 $\pm$ 2.1	46.6 $\pm$ 2.3	$4.6 \times 10^{-3} \pm 5.2 \times 10^{-4}$	$4.6 \times 10^{-3} \pm 9.2 \times 10^{-4}$	$4.0 \times 10^{-5} \pm 8.2 \times 10^{-6}$	$3.8 \times 10^{-5} \pm 1.6 \times 10^{-5}$
1.0 $\mu\text{M}$ NU172	68.6 $\pm$ 3.4	71.7 $\pm$ 4.9	$4.4 \times 10^{-3} \pm 8.4 \times 10^{-4}$	$3.6 \times 10^{-3} \pm 8.0 \times 10^{-4}$	$2.1 \times 10^{-5} \pm 1.4 \times 10^{-6}$	$2.1 \times 10^{-5} \pm 5.5 \times 10^{-6}$
2.0 $\mu\text{M}$ NU172	>600	>600	$1.9 \times 10^{-3} \pm 2.4 \times 10^{-4}$	$5.4 \times 10^{-3} \pm 2.3 \times 10^{-4}$	$3.4 \times 10^{-6} \pm 3.1 \times 10^{-6}$	$1.1 \times 10^{-6} \pm 3.1 \times 10^{-5}$
1.0 $\mu\text{M}$ NS_AD	47.0 $\pm$ 3.0	47.1 $\pm$ 1.3	$5.6 \times 10^{-3} \pm 1.0 \times 10^{-3}$	$6.0 \times 10^{-3} \pm 7.8 \times 10^{-4}$	$2.8 \times 10^{-5} \pm 1.3 \times 10^{-5}$	$4.9 \times 10^{-5} \pm 1.6 \times 10^{-5}$
1.0 $\mu\text{M}$ AD	46.2 $\pm$ 1.8	46.8 $\pm$ 1.3	$4.5 \times 10^{-3} \pm 6.1 \times 10^{-4}$	$5.8 \times 10^{-3} \pm 7.6 \times 10^{-4}$	$3.0 \times 10^{-5} \pm 3.3 \times 10^{-6}$	$4.3 \times 10^{-5} \pm 1.4 \times 10^{-5}$

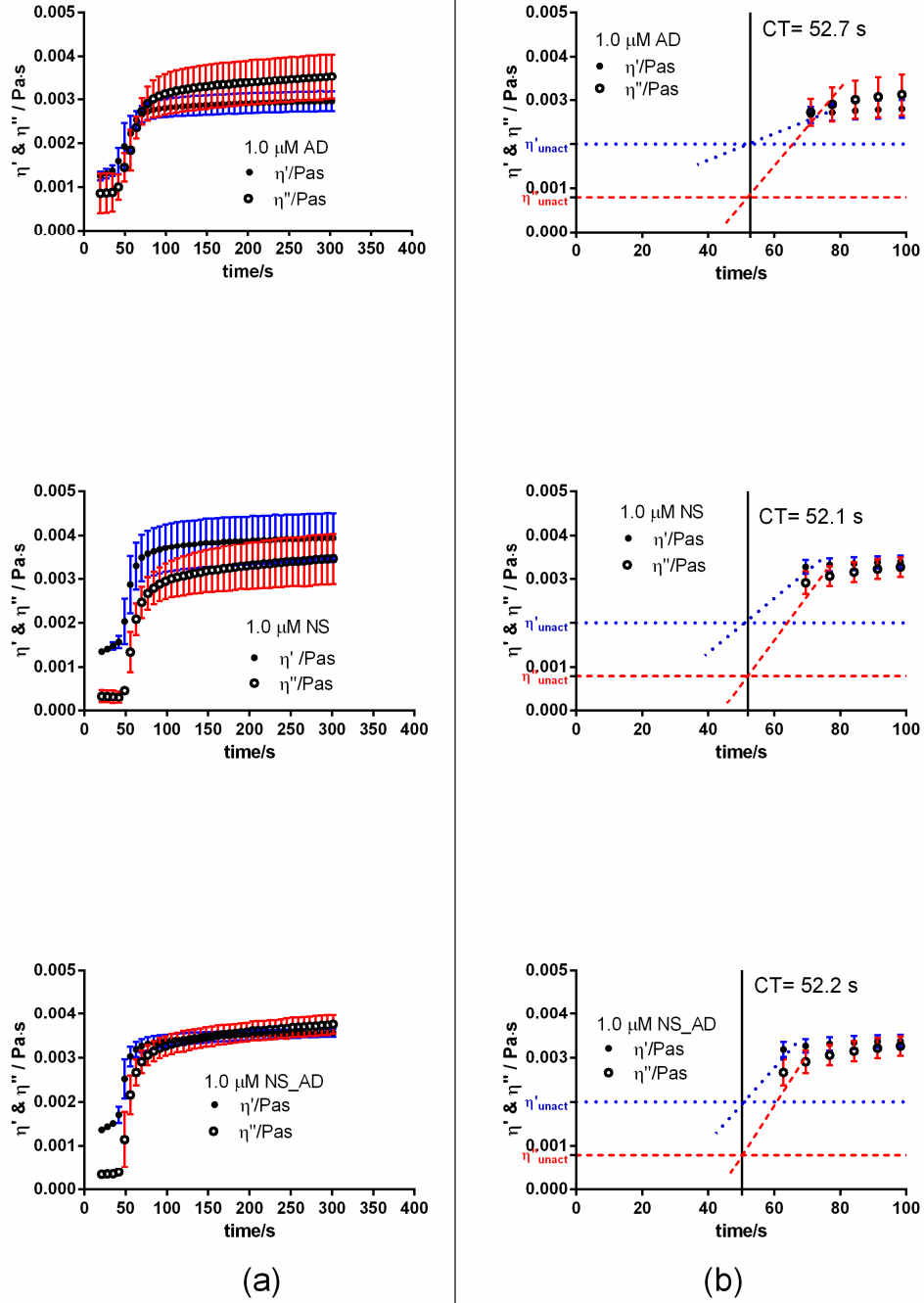
<sup>1</sup>. clotting time (CT), viscous component ( $\eta'$ ), elastic component ( $\eta''$ ), the linear slope of the viscous or elastic component (m), thrombin aptamer (NU172), antidote (AD), nonsense antidote (NS\_AD)

**Table S4.** Heparinized blood was incubated for 2 min with 1.0  $\mu\text{M}$  NU172, then 1.0  $\mu\text{M}$  AD was added to the NU172 aptamer containing blood and incubated for 5 min. The clotting time (CT) was determined before and after the circulation for 30 min at 37  $^{\circ}\text{C}$  in an in vitro rotation model using PIEZ or KC 1A. Blood without any additives was indicated as a baseline. Blood with NaCl, 1.0  $\mu\text{M}$  NS\_AD, or AD were used as negative controls. Blood with 1.0  $\mu\text{M}$  NU172 served as positive control. Blood from five different volunteers was used ( $n = 5 \pm \text{SD}$ ). Using PIEZ, the clotting time (CT), viscous ( $\eta'$ ) and elastic ( $\eta''$ ) components at 300 s, and linear slopes ( $m(\eta')$  and  $m(\eta'')$ ) were measured <sup>1</sup>.

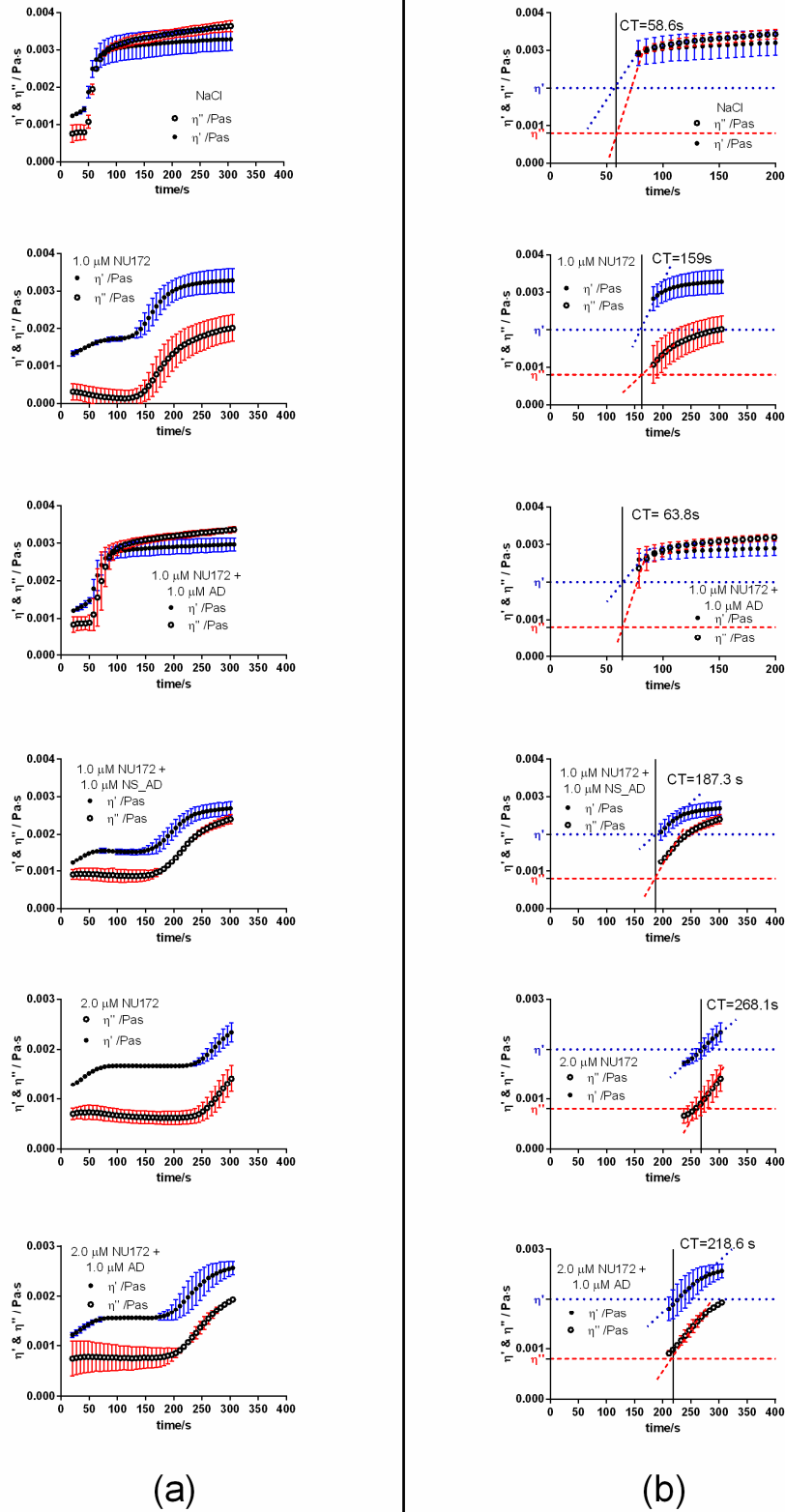
Sample	PIEZ	KC 1A	PIEZ			
	CT/s	CT/s	$\eta'$ (Pa s) at 300 s	$\eta''$ (Pa s) at 300 s	$m(\eta'/\text{Pa})$	$m(\eta''/\text{Pa})$
<b>Baseline</b>	45.9 $\pm$ 1.2	45.7 $\pm$ 2.0	$8.1 \times 10^{-3} \pm 3.8 \times 10^{-3}$	$1.2 \times 10^{-2} \pm 4.0 \times 10^{-3}$	$5.7 \times 10^{-5} \pm 2.0 \times 10^{-5}$	$3.4 \times 10^{-4} \pm 3.3 \times 10^{-4}$
<b>0 min</b>						
NaCl	45.5 $\pm$ 1.2	45.7 $\pm$ 2.0	$5.7 \times 10^{-3} \pm 2.6 \times 10^{-4}$	$9.4 \times 10^{-3} \pm 2.5 \times 10^{-3}$	$4.8 \times 10^{-5} \pm 5.4 \times 10^{-6}$	$1.8 \times 10^{-4} \pm 8.2 \times 10^{-5}$
1.0 $\mu\text{M}$ NS_AD	47.6 $\pm$ 0.9	47.2 $\pm$ 1.8	$8.2 \times 10^{-3} \pm 1.1 \times 10^{-3}$	$1.0 \times 10^{-2} \pm 3.1 \times 10^{-3}$	$8.9 \times 10^{-5} \pm 2.8 \times 10^{-5}$	$2.7 \times 10^{-4} \pm 8.1 \times 10^{-5}$
1.0 $\mu\text{M}$ AD	46.7 $\pm$ 1.3	47.7 $\pm$ 4.2	$7.0 \times 10^{-3} \pm 3.6 \times 10^{-3}$	$1.0 \times 10^{-2} \pm 5.5 \times 10^{-3}$	$7.5 \times 10^{-5} \pm 5.2 \times 10^{-5}$	$2.3 \times 10^{-4} \pm 1.6 \times 10^{-4}$
1.0 $\mu\text{M}$ NU172	> 600	> 600	$4.2 \times 10^{-3} \pm 5.6 \times 10^{-4}$	$2.2 \times 10^{-3} \pm 9.0 \times 10^{-4}$	$1.1 \times 10^{-5} \pm 3.2 \times 10^{-6}$	$8.1 \times 10^{-6} \pm 1.9 \times 10^{-6}$
1.0 $\mu\text{M}$ NU172 + 1.0 $\mu\text{M}$ AD	51.9 $\pm$ 4.6	57.9 $\pm$ 11.4	$5.7 \times 10^{-3} \pm 4.6 \times 10^{-4}$	$7.3 \times 10^{-3} \pm 7.7 \times 10^{-4}$	$3.9 \times 10^{-5} \pm 2.0 \times 10^{-5}$	$1.2 \times 10^{-4} \pm 7.2 \times 10^{-5}$
<b>After 30 min</b>						
NaCl	45.3 $\pm$ 0.9	44.0 $\pm$ 4.2	$4.5 \times 10^{-3} \pm 1.6 \times 10^{-3}$	$6.7 \times 10^{-3} \pm 3.3 \times 10^{-3}$	$3.1 \times 10^{-5} \pm 1.5 \times 10^{-5}$	$8.7 \times 10^{-5} \pm 5.2 \times 10^{-5}$
1.0 $\mu\text{M}$ NS_AD	47.7 $\pm$ 1.4	46.4 $\pm$ 1.2	$6.1 \times 10^{-3} \pm 1.5 \times 10^{-3}$	$8.3 \times 10^{-3} \pm 1.2 \times 10^{-3}$	$5.2 \times 10^{-5} \pm 3.4 \times 10^{-5}$	$1.4 \times 10^{-4} \pm 1.1 \times 10^{-4}$
1.0 $\mu\text{M}$ AD	46.7 $\pm$ 1.6	45.3 $\pm$ 4.9	$7.8 \times 10^{-3} \pm 3.4 \times 10^{-3}$	$9.9 \times 10^{-3} \pm 4.4 \times 10^{-3}$	$8.8 \times 10^{-5} \pm 6.0 \times 10^{-5}$	$2.5 \times 10^{-4} \pm 1.6 \times 10^{-4}$
1.0 $\mu\text{M}$ NU172	> 600	> 600	$4.4 \times 10^{-3} \pm 5.5 \times 10^{-4}$	$2.3 \times 10^{-2} \pm 9.9 \times 10^{-4}$	$1.5 \times 10^{-5} \pm 2.6 \times 10^{-6}$	$9.6 \times 10^{-6} \pm 2.4 \times 10^{-6}$
1.0 $\mu\text{M}$ NU172 + 1.0 $\mu\text{M}$ AD	49.3 $\pm$ 2.0	61.6 $\pm$ 3.0	$5.3 \times 10^{-3} \pm 1.4 \times 10^{-3}$	$6.1 \times 10^{-3} \pm 1.0 \times 10^{-3}$	$3.1 \times 10^{-5} \pm 1.4 \times 10^{-5}$	$6.1 \times 10^{-5} \pm 5.2 \times 10^{-6}$

<sup>1</sup>. clotting time (CT), viscous component ( $\eta'$ ), elastic component ( $\eta''$ ), the linear slope of the visocus or elastic component (m), thrombin aptamer (NU172), antidote (AD), nonsense antidote (NS\_AD)

## Supplementary Figures



**Figure S1.** Detection of clotting time (CT) in citrated blood using PIEZ after the addition of 1.0  $\mu\text{M}$  AD, NS, or NS\_AD. (a) Detection of mean viscous ( $\eta'$ ) and elastic ( $\eta''$ ) components of blood at 100 Hz and 37  $^{\circ}\text{C}$  ( $n = 10 \pm \text{SD}$ ). (b) Enlarged parts of diagrams for the calculation of CT. The horizontal dotted blue line at 0.002 Pa·s visualizes the  $\eta'$  and the dashed red line at 0.0008 Pa·s visualizes the  $\eta''$  of unactivated citrated blood.



**Figure S2.** Detection of clotting time (CT) in citrated blood using PIEZ. After 2 min of incubation with 1.0 or 2.0  $\mu\text{M}$  NU172, 1.0  $\mu\text{M}$  AD or 1.0  $\mu\text{M}$  NS\_AD was added. Furthermore, CT was detected in blood containing NaCl, 1.0, or 2.0  $\mu\text{M}$  NU172. (a) Detection of mean viscous ( $\eta'$ ) and elastic ( $\eta''$ ) components of blood at 100 Hz and 37 °C ( $n = 5 \pm \text{SD}$ ). (b) Enlarged parts of diagrams for the calculation of CT. The horizontal dotted blue line at 0.002 Pa.s visualizes the  $\eta'$  and the dashed red line at 0.0008 Pa.s visualizes the  $\eta''$  of unactivated citrated blood.